

Listing of claims (8 March 2011)

1. (currently amended) A method for determining the presence of genetic element(s), in a nucleic acid sample, which method comprises the steps of:

- a) providing the nucleic acid sample comprising the genetic element(s);
 - b) providing oligonucleotide(s) that are completely or partially complementary to, but that are out of phase with, the region(s) comprising the genetic element(s) of said nucleic acid sample;
 - c) annealing said oligonucleotide(s) to said nucleic acid sample;
 - d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme; and
 - e) detecting pyrophosphate to determine whether a ligation reaction has occurred, as a measure of the presence of the genetic element(s),
- wherein steps a)-e) are performed simultaneously or subsequently or in any combination of subsequent steps.

2. (currently amended) A method for analysing the number of nucleotide repeats in a nucleic acid sample, which method comprises the steps of:

- a) providing the nucleic acid sample potentially comprising a nucleotide repeat;
- b) providing oligonucleotide(s) complementary to, but that are out of phase with, said nucleotide repeat;
- c) annealing said oligonucleotide(s) to said nucleic acid sample;
- d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme; and
- e) detecting pyrophosphate to determine whether a ligation reaction has occurred, wherein steps a)-e) are performed simultaneously or subsequently or in any combination of subsequent steps.

3. (currently amended) A method for analysing the number of nucleotide repeats in a nucleic acid sample, which method comprises the steps of:

- a) providing the nucleic acid sample potentially comprising a nucleotide repeat;
- b) providing oligonucleotide(s) complementary to, but that are out of phase with, said nucleotide repeat;

- c) annealing said oligonucleotide(s) to said nucleic acid sample;
 - d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme;
 - e) converting pyrophosphate into ATP; and
 - f) detecting said ATP to determine whether a ligation reaction has occurred, wherein steps a)-f) are performed simultaneously or subsequently or in any combination of subsequent steps.
4. (currently amended) A method for analysing the number of nucleotide repeats in a nucleic acid sample, which method comprises the steps of:
- a) providing the nucleic acid sample potentially comprising a nucleotide repeat;
 - b) providing oligonucleotide(s) complementary to, but that are out of phase with, said nucleotide repeat;
 - c) annealing said oligonucleotide(s) to said nucleic acid sample;
 - d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme;
 - e) converting pyrophosphate into ATP; and
 - f) detecting said ATP by a luciferase-based assay as a measure of whether a ligation reaction has occurred, wherein steps a)-f) are performed simultaneously or subsequently or in any combination of subsequent steps.
5. (currently amended) A method for microbial typing of a nucleic acid sample, which method comprises the steps of:
- a) providing the nucleic acid sample comprising at least one marker for microbial typing;
 - b) providing oligonucleotide(s) that are completely or partially complementary to, but that are out of phase with, the region(s) comprising marker(s) for microbial typing of said nucleic acid sample;
 - c) annealing said oligonucleotide(s) to said nucleic acid sample;
 - d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme; and
 - e) detecting pyrophosphate to determine whether a ligation reaction has occurred;

- f) comparing the ligation pattern of the sample with a reference pattern, in order to determine the microbial type,
wherein steps a)-e) are performed simultaneously or subsequently or in any combination of subsequent steps.
6. (previously presented) The method according to any one of claims 1-5 wherein one of the oligonucleotides in step b) is adapted to anneal immediately outside the repeated sequence.
 7. (cancelled)
 8. (previously presented) The method according to any one of claims 1-7 wherein step d) is performed employing a NAD^+ -dependent DNA-ligase.
 9. (previously presented) The method according to any one of claims 1-8 wherein step e) is performed employing a pyruvate phosphate dikinase.
 10. (previously presented) The method according to any one of claims 1-6, wherein step d) is performed employing an ATP-dependent ligase, and apyrase is added to the ligation mixture of step d) before, during or after ligation in order to reduce excess amounts of DNA ligase substrate.
 11. (previously presented) The method according to claim 10, wherein the ATP dependent ligase is T4 DNA ligase.
 12. (previously presented) The method according to claim 10 or 11, wherein dATP is used as a substrate for the ATP dependent ligase in step d).
 13. (cancelled)
 14. (previously presented) The method according to any one of claims 1-6 or 10-13, wherein step e) is performed employing a ATP-sulfurylase.
 15. (previously presented) The method according to any one of claims 1-14, wherein the oligonucleotide employed is a mono-, di- or multimer of the repeat in itself.
 16. (cancelled)
 17. (previously presented) The method according to any one of claims 1-15, further comprising a step wherein unannealed oligonucleotides are removed after the detection by using an exonuclease.

18. (previously presented) The method according to any one of claims 1-15, further comprising a step wherein unannealed oligonucleotides are inactivated after the detection by using a phosphatase.

19. (previously presented) The method according to any one of claims 1-18, wherein the nucleic acid sample is immobilised on a support.

20. (previously presented) The method according to claim 19, further comprising a step wherein unannealed oligonucleotides are removed after the detection by washing.

21. (previously presented) The method according to any one of claims 1-20, preceded by a step wherein the nucleic acid sample is amplified.

22. (previously presented) The method according to any one of claims 1-21, wherein the luciferase-based assay is a luminometric assay.

23. (previously presented) The method according to any one of claims 1-22, wherein the light that is produced in the luciferase reaction is enzymatically turned off after an initial level of produced light has been reached.

24. (previously presented) The method according to claim 23, wherein light production is turned off by the addition of apyrase.

25. (previously presented) The method according to any one of claims 1-24 where oligonucleotides complementary to a region outside that to be analyzed are used to generate a signal by ligation or primer extension that can be used to normalize the signal obtained from the region to be analyzed.

26-34. (cancelled)